Chimerism Testing Using VNTR/STR Markers

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Disclosure Information

I have no relevant conflicts of interest to declare.

Hematopoetic Stem Cell Transplant (HSCT)

- Deficiencies in red and white blood cells
  - Aplastic anemia, SCID, CN
- Excessive production of blood cells
  - Leukemia or lymphoma
- Functional defects in blood cells
  - Thalassemia, sickle cell disease
Sources of Hematopoetic Stem Cells
Related or Unrelated Donors

- Bone marrow (BM)
- Peripheral blood stem cells (PBSC)
- Umbilical cord blood
  - Advantage: Less stringent HLA matching increasing donor availability.
  - Disadvantage: Cell count significantly lower than BM or PBST leading to delayed engraftment and an increase in graft failure.

Using More Than One Cord-Blood Donor

Immune system attack can be monitored in T-cells (CD3+)
Clinical Utility of Chimerism Detection

- Monitor engraftment process
- Detects graft failure
- Rules out graft failure
- Risk of relapse
- Graft vs host disease (GVSD)
- Response to therapies (such as DLI)

Detecting Chimerism Post-Transplant

- Genetic differences between individuals
- Polymorphisms are sequence variations that are common in a population.
  - Single nucleotide polymorphisms (SNP)
  - Copy number variants (CNV)
- Female (XX) vs Male (XY)

Tandem Repeat Polymorphisms

- Highly polymorphic loci
- Allelic differences in the number of repeats
- Many loci throughout the genome
- Genetic markers for identity testing
- Sensitive markers for detecting chimerism
Tandem Repeat Polymorphisms

VNTR vs STR

- Variable number of tandem repeats (VNTR)
  Repeat size 5-100 bp
  Many alleles
  PCR and Southern Blot

- Small tandem repeats (STR), microsatellites
  Repeat size 2-4 bp
  Many loci, many alleles
  Amenable to PCR

Properties of VNTRs/STRs

- Genetic locus with typical inheritance
- Two copies per genome: one maternal, one paternal
- Many VNTR/STR loci have been identified on different chromosomes
- Best loci have many alleles and a high %heterozygosity

VNTR Loci For Engraftment Testing

<table>
<thead>
<tr>
<th>Loci</th>
<th>Chromosome</th>
<th>Size (bp)</th>
<th># Alleles</th>
<th>% Het.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoB</td>
<td>2</td>
<td>30</td>
<td>&gt;12</td>
<td>80</td>
</tr>
<tr>
<td>D1S80</td>
<td>1</td>
<td>16</td>
<td>&gt;29</td>
<td>79</td>
</tr>
<tr>
<td>D17S5</td>
<td>17</td>
<td>70</td>
<td>&gt;12</td>
<td>84</td>
</tr>
<tr>
<td>33.6</td>
<td>1</td>
<td>37</td>
<td>&gt;13</td>
<td>67</td>
</tr>
</tbody>
</table>
Informative Analysis

- Identify a genetic locus that differentiates recipient and donor
- Requires pre-transplant sample from recipient and donor
- If pre-transplant recipient sample not available, a buccal swab can be used.
Calculations: Single Donor

\[
\% \text{Recipient} = \frac{\text{RSA}}{\text{RSA} + \text{DSA} \text{ (Total area)}} \times 100%
\]

\[
\% \text{Donor} = 100\% - \% \text{Recipient}
\]

RSA = Recipient Specific allele area
DSA = Donor Specific allele area

Calculations: Two Donors

\[
\% \text{Recipient} = \frac{\text{RSA}}{\text{RSA} + \text{D1SA} + \text{D2SA} \text{ (Total area)}} \times 100%
\]

\[
\% \text{Donor} 1 = \frac{\text{D1SA}}{\text{RSA} + \text{D1SA} + \text{D2SA} \text{ (Total area)}} \times 100%
\]

\[
\% \text{Donor} 2 = 100\% - \% \text{Recipient} - \% \text{Donor} 1
\]

RSA = Recipient Specific allele area
D1SA = Donor 1 Specific allele area
D2SA = Donor 1 Specific allele area

Post-Transplant Chimerism Analysis

Recipient

92% Donor
8% Recipient

Donor

\[
\% \text{Recipient} = \frac{\text{R(R-D)}}{\text{R(R-D) + 3997} \times 100\%} = 3997 \times 100\% = 8\%
\]

\[
\% \text{Donor} = 100\% - \% \text{R} = 100\% - 8\% = 92\%
\]
Chimerism Analysis:
CD3+ and CD33+ Cell Populations

Recipient

Donor

CD3+ cells 35% recipient

CD33+ cells 67% recipient

%Recipient = Recipient / Recipient + donor

Double Cord Pre-Transplant testing

Recipient

Donor 1

Donor 2

Triple Cord Transplant

Recipient

Donor 1

Donor 2

Donor 3
Multiplex STR Assay

Informative Allele Determination

<table>
<thead>
<tr>
<th>Locus</th>
<th>Recipient Alleles</th>
<th>Donor 1 Alleles</th>
<th>Donor 2 Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>D5S818</td>
<td>11, 12</td>
<td>12, 12</td>
<td>11, 11</td>
</tr>
<tr>
<td>D13S317</td>
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<tr>
<td>D7S820</td>
<td>9, 10</td>
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<td>D16S539</td>
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<td>vWA</td>
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<td>FTOX</td>
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<td>CSF1PO</td>
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<tr>
<td>F13B</td>
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<td>8, 10</td>
<td>10, 10</td>
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<tr>
<td>FESFPS</td>
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<td>10, 12</td>
<td>10, 11</td>
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<tr>
<td>F13A01</td>
<td>8, 10</td>
<td>8, 10</td>
<td>10, 10</td>
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<tr>
<td>Amelogenin</td>
<td>X, Y</td>
<td>X, Y</td>
<td>X, X</td>
</tr>
</tbody>
</table>

One locus or a combination can be used to calculate chimerism.

Sensitivity of Detection

- The sensitivity indicates the lowest percent of recipient or donor cells detected by the assay.
- Recipient or donor cells below this amount would not be detected.
- Sensitivity is important to consider when sample is 100% recipient or donor.
Sensitivity of Detection: Examples

- Sample reported as 100% donor with 10% sensitivity means that recipient cells below 10% will not be detected. This may or may not be clinically significant.
- Sample is reported as 50% recipient and 50% donor with 20% sensitivity. Since chimerism is detected, the low assay sensitivity is irrelevant.

How is Sensitivity Determined?

- Determine the major peak area required to detect 1, 2, 5 and 10%.
- Number of cells in the sample may limit the sensitivity. Commonly occurs early in transplant.
- Monoplex assays typically have better sensitivity than multiplex assays.

Sample Report